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Physiological Evaluation of the Role of the Liver as a Mediator of the Growth-Promoting Action of Somatotrophin

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ABSTRACT—The role of the liver as a mediator of the somatotrophic actions of GH was investigated in three experiments in hypophysectomized rats. The GH was infused via osmotic minipumps into the hepatic portal vein (HPV) or the external jugular vein (EJV) for 7 days at a constant rate or in 8 pulses of 1 hour each/day. Infusion of rat (r) GH at 1 μg/rat/day into the two vessels in rats hypophysectomized one day after catheterization failed to affect body growth, but it increased their tibial epiphysial plate width (TEPW), and serum IGF-I concentration to the same degree regardless of the site of delivery or the schedule of infusion. When bovine (b) GH was infused at (50 μg/rat/day) starting 12–14 days after hypophysectomy, the EJV route was more effective at stimulating growth and elevating serum IGF-I than was HPV infusion by both modes of delivery, and constant infusion was more effective than pulsed delivery. In the third experiment human (h) GH infusion was started 30 days after hypophysectomy at 50 μg/rat/day. This treatment caused much more striking growth responses than did either of the other two GH preparations, and the EJV route of delivery was again more effective than was infusion into the HPV by both schedules. In addition, the constant mode of delivery was again more effective than was pulsed infusion. These results indicate that effects of GH on peripheral tissues may be more important for growth than effects mediated by the liver. Thus, the hepatic effects of GH may involve maintaining the responsiveness of the liver to other regulators of IGF-I secretion.

INTRODUCTION

The somatomedin hypothesis [1, 2] proposed that growth hormone (GH) acts indirectly to promote growth by stimulatng hepatic production of a mediator, which is now known as insulin-like growth factor-I (IGF-I). Numerous observations support this hypothesis. For example, several groups have reported that GH increases the secretion of somatomedin/IGF-I by the perfused rat liver and by hepatic explants or cells in culture [see reviews in 3]. In addition, *in vivo* studies involving partial hepatectomy [4], cross-hepatic blood sampling [5] and studies of patients with liver disorders [6] demonstrated that the liver produces IGF-I, and that this production is stimulated by GH. Furthermore, Schwander *et al.* [7] estimated that hepatic production of IGF-I in response to GH can account for all of the IGF-I in serum.

Other data indicate that GH can also act peripherally [8–14]. These results raise questions about the relative significance of peripheral *vs* hepatic actions of GH in promoting somatic growth. Mick and Nicoll [15] developed a technique for testing the direct effects of hormones on hepatic functions *in vivo*. Their system involves chronic infusion into the hepatic portal vein, which delivers hormone directly to the liver via one of its two blood supplies. Thus, the direct effect of substances on hepatic functions *in vivo* can be studied in a physiologically meaningful way. In the present study we used their procedure [15] to reevaluate the basic tenet of the somatomedin hypothesis [1, 2].

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MATERIALS AND METHODS

Animals

Male rats weighing 120–150 gm were obtained from our own breeding colony or from Simonsen Laboratories (Gilroy, CA, U.S.A.) and maintained as described previously [10]. They were hypophysectomized (Hx) by a transauricular approach [16] either before or after catheterization, depending upon the schedule of the experiment. All procedures used on the rats were described in detail in a protocol that was approved by our Institutional Animal Care and Use committee, and all experiments conformed to the regulations described in the N.I.H. Guide to the Care and Use of Laboratory Animals.

Catheterization

The procedures used to construct the catheters and insert them into either the hepatic portal vein (HPV) or the external jugular vein (EJV) and connecting them to osmotic minipumps were as described [10, 13]. In brief, for constant infusion, Alzet minipumps (2001: Alza Corp, Palo Alto, CA, U.S.A.) were filled with solvent [17] with or without GH, and a 7-cm long catheter filled with solvent containing heparin was attached. For pulsatile delivery, the polyethylene 50 catheter was extended to 70 cm and 1-mm segments of solvent or GH solution were drawn into it. The segments were separted by 2-mm-long air bubbles. By this means the GH solution or solvent was infused in 8 pulses/day of about 1 hour duration with approximately a 2-hour interpulse interval. This mode of delivery should mimic the endogenous GH secretion pattern in adult male rats [18].

Measurements

Changes in body weight were recorded in all three experiments and changes in tail length were measured in two of them. These measurements were made only at the beginning and the end of each experiment. The rats were anesthetized and blood was drawn by cardiac puncture and the tibiae were removed and processed for measurement of the width of the epiphysial cartilage plate [TEPW; 19]. The sellar region was examinced carefully using a binocular dissecting microscope for the presence of pituitary remnants. Animals with detectable remnants and/or lack of obvious testicular regression were judged to be incompletely hypophysectomized and were excluded.

Hormones and Assays

Serum from the rats used in the first two experiments was processed for measurement of IGF-I by RIA and the concentration of GH was measured by RIA in the serum samples from the rats that received constant infusion of the (r)GH. Details of these RIA procedures have been published [20, 21]. However, because of the breakdown of a freezer, some of the serum samples from the three experiments were lost and so they could not be assayed for IGF-I, human (h)GH or bovine (b)GH levels. The three preparations of GH used were obtained from the National Hormone and Pituitary Program of the NIH (USA). The rGH (NIH B-9), bGH (NIH B-18), and hGH (NIDDK B-1) had potencies of 1.9, 3.2 and 2.4 I.U./mg, respectively.

Experiments

Three experiments were conducted—one each with rat (r), bovine (b), and human (h) GH. In the first study the rats were Hx one day after catheterization and were killed 6 days later. Thus, this experiment was designed to test the effectiveness of the rGH at maintaining growth after pituitary removal. The dose of rGH of 1 μg/rat/day was selected on the basis of results of our previous study [10] in which rGH was infused into the arterial supply of one hindlimb of Hx rats. A dose of 2 μg/rat/day caused a striking increase in the TEPW in the infused limb but it caused a significant, though lesser growth effect in the contralateral hindlimb. Thus, the 1 μg/day dose given via the HPV should have a significant effect on hepatic secretion of IGF-I and growth while the same dose given into the EJV should have a lesser effect.

In the second experiment, the rats were Hx 12–14 days prior to catheterization and bGH was infused for 7 days at a dose of 50 μg/rat/day to obtain larger responses. In the third experiment, the rats were Hx 30 days before catheterization

and bGH was infused at a dose of 50 μg/rat/day for 7 days. In these experiments with rGH and hGH the rats were killed on the 8th day after the placement of the catheters and minipumps. In the experiments with rGH and hGH control rats received infusion of the solvent that was used to dissolve the GH preparations. In the experiment with bGH the abdomen of the controls was opened to expose the intestines, but no catheter was inserted into an intestinal vein. In all three experiments the rats were killed on the 8th day after the start of infusion.

Significance of differences was determined by the Student's t test or by analysis of variance, as described [10].

RESULTS

In the experiment with rat GH, all of the experimental groups showed an equivalent loss in body weight and reduction in tail growth relative to intact or sham Hx controls. Infusion of the rGH did not affect these changes in body weight or tail length in any of the experimental groups (data not shown). Serum GH was not detectable by RIA (sensitivity of 0.7 ng/ml) in any of the rats that received solvent infusions. In those given constant infusion of the hormone via the EJV or the HPV, the serum GH concentrations were 3.0 ± 0.4 ng/ml (n=7) and 3.2 ± 0.1 ng/ml (n=5), respectively. Thus, the route of administration did not affect the peripheral concentration of GH. Serum GH levels were not measured in the rats that received pulse infusion because meanigful data from these animals could be obtained only by taking multiple samples over an extended period.

Intact rats of the same age as those used in these experiments had a mean TEPW of 314 μm. Thus, the data in Figure 1 show that the TEPW in the rats infused with the solvent had regressed by more

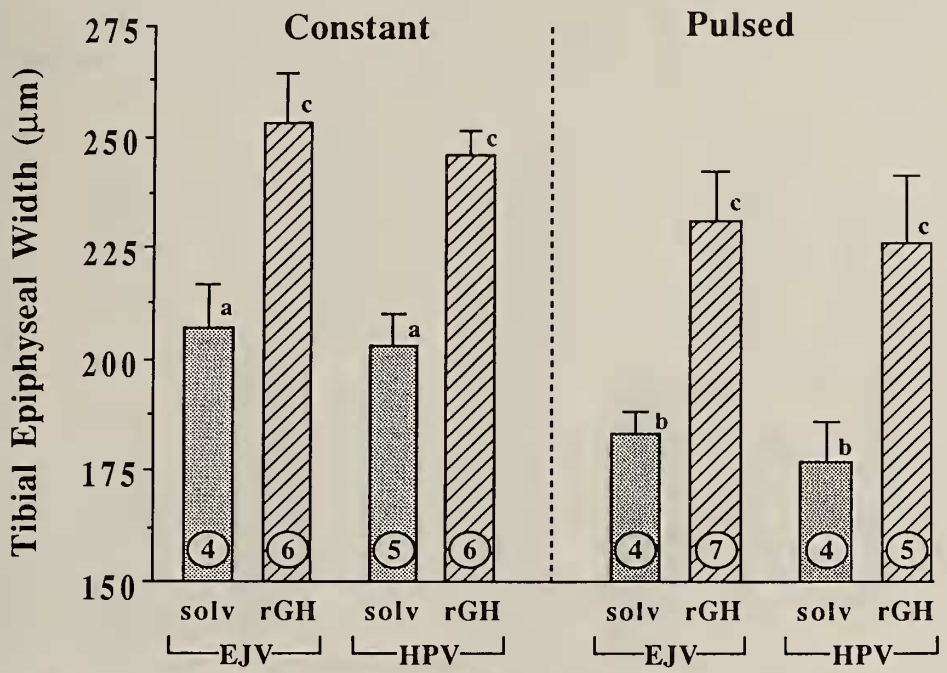

FIG. 1. Effect of infusing solvent (solv) or rGH (1 μg/rat/day) in solvent into the external jugular vein (EJV) or hepatic portal vein (HPV) on the tibial epiphysial plate width of hypophysectomized rats for 7 days. The rats were Hx one day after placement of the catheters and the infusions were either constant or pulsatile (8 pulses of 1 hour each). The number of rats in each group is given within the base of each column. Columns with the same letter superscript are not significantly different from each other. Those with different letters are significant at $P < 0.001$. The columns represent the mean $\pm$ SEM values for each group.

than 100 μm during the 6 days following hypophysectomy. The TEPW of the rats that received the solvent in pulses was slightly lower than those measured in the animals given constant infusions. This difference is probably due to the fact that the two groups of experiments (constant and pulsed infusions) were done at different times.

The data in Figure 1 show that constant delivery of rGH into the EJV or the HPV increased the mean TEPW over that in the controls given solvent to the same extent [i.e. by 46 μm ($P<0.01$), and 43 μm ($P<0.001$), respectively]. When the rGH was infused in pulses into the EJV or the HPV, the degree of growth stimulation (+48 μm in both groups, $P<0.01$) was similar to that produced by constant infusion. Thus, intrahepatic delivery of the hormone either constantly or in pulses was no more effective than infusing it into the peripheral vessel.

The rats infused with solvent at a constant rate had mean serum IGF-I levels of 55–57 μg/ml, regardless of the site of infusion (Fig. 2). This concentration is less tha 10% of the level measured in intact rats of the same age (i.e. 615 μg/ml). Infusion of rGH into either the HPV or the EJV elevated serum IGF-I concentrations to the same degree over those in the rats that were infused with solvent. Thus, the serum IGF-I levels in these animals are consistent with their equivalent TEPW measurements (Fig. 1) and serum concentrations of rGH.

The effects of infusing bGH at 50 μg/rat/day on changes in body weight and tail length are shown in Fig. 3. The solvent-infused rats lost about 10 gm in body weight during the 7-day experimental period but constant infusion of bGH into either vein prevented this loss. However, constant delivery into the EJV was significantly more effective than was infusion into the HPV. Pulsed delivery of bGH into the HPV did not prevent the loss in body weight. Although pulsed infusion of GH into the EJV was effective in this regard, it was significantly less effective than was constant infusion into that vein. Tail growth was increased significantly only in the groups that received bGH constantly via the EJV.

Only the group that received constant infusion of bGH into the EJV showed a singificant TEPW

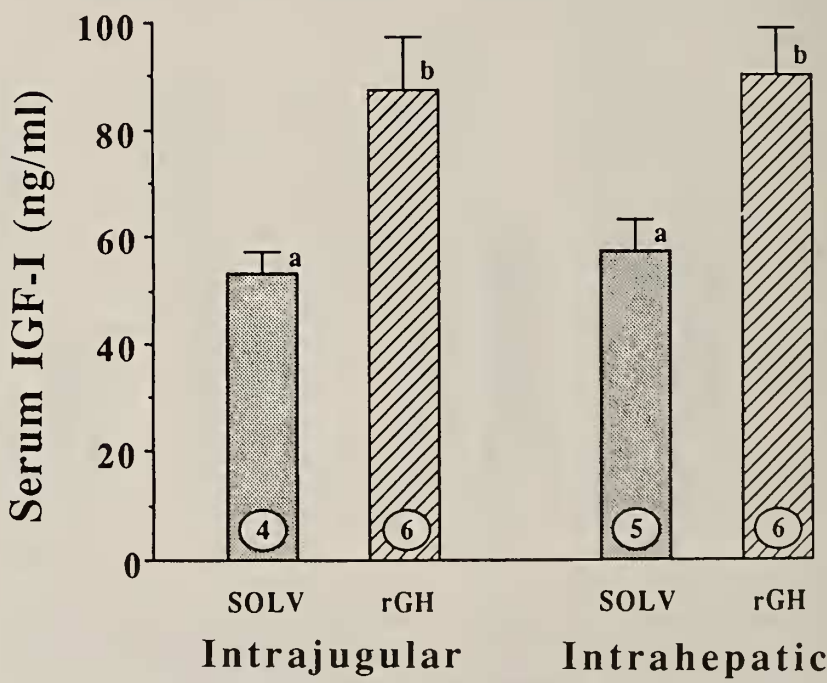

Fig. 2. Serum IGF-I levels in the rats that received constant infusions as described in Fig. 1. Otherwise as in the legend for Fig. 1, but columns with different letters as significantly different at $P<0.01$.

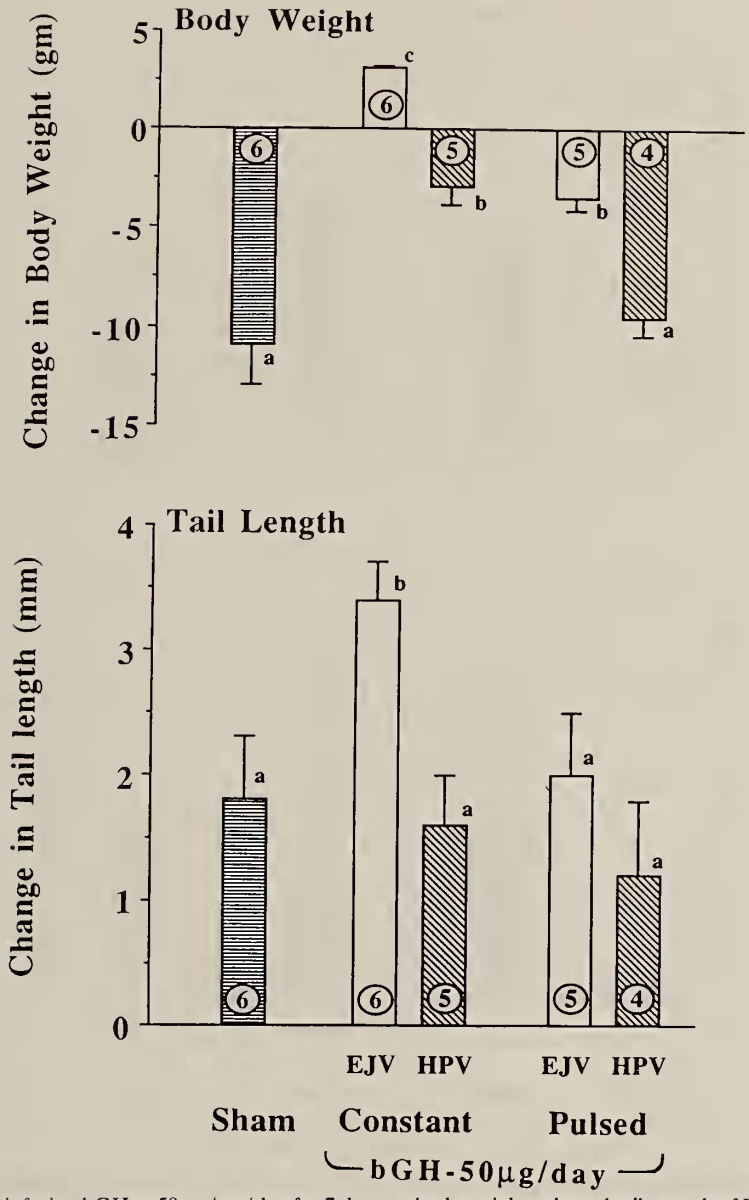

FIG. 3. Effects of infusing bGH at 50 μg/rat/day for 7 days on body weight gain and tail growth of Hx rats. Infusions were started 12–14 days after Hx and the bGH was given constantly or in pulses, as described in Fig. 1. The sham operated rats were subjected to the same surgical procedures as those that were catherized for intrahepatic infusions but without placement of either a catheter or minipumps. Columns with different letters are signicficantly different at $P<0.05$ or better.

response to the hormone (Fig. 4). However, serum IGF-I levels were significantly elevated in the rats given constant infusion of bGH via either the EJV or the HPV (2.5-fold and 2.1-fold, respectivley). Thus, in this study, serum IGF-I levels were not completely consistent with the other growth parameters measured. In the third experiment, the rats that received solvent infusion into either the HPV or the EJV starting 30 days after pituitary ablation lost about 5 gm in body weight

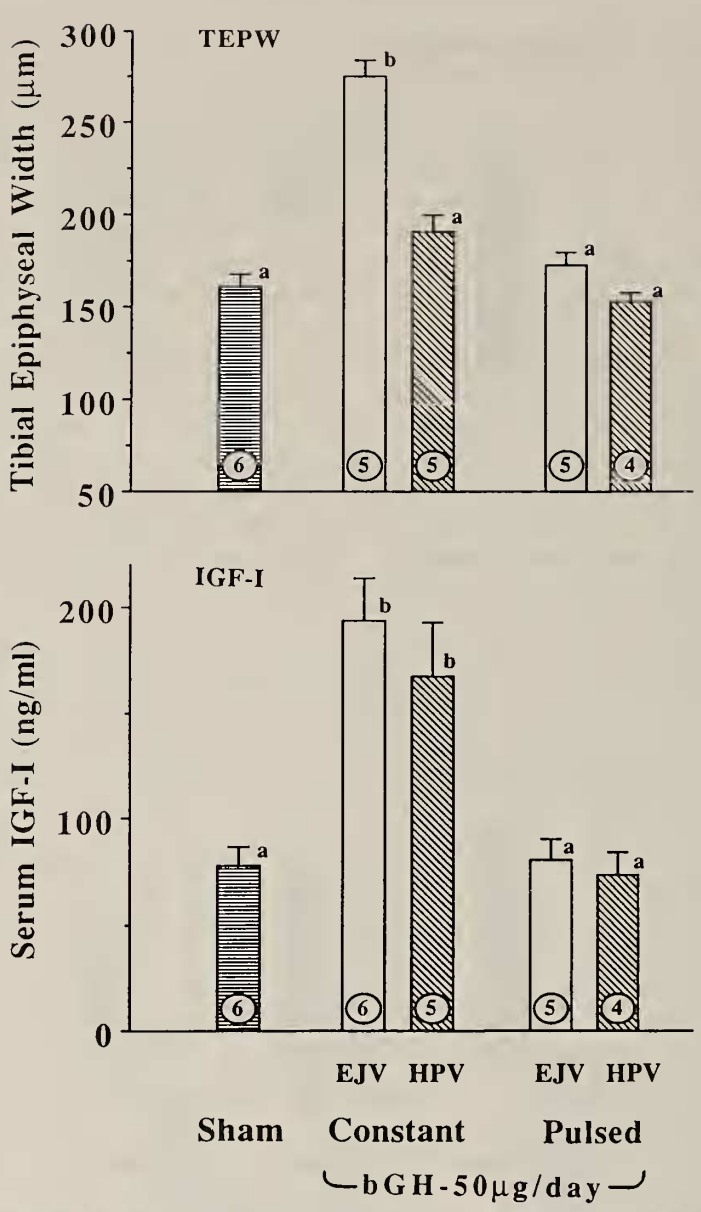

FIG. 4. Tibial epiphysial plate widths and serum IGF-I levels in the rats described in the legend of Fig. 3. Columns with different letter superscripts are significantly different from each other at $P<0.001$.

during the 7-day experimental period (Fig. 5). Constant infusion of hGH into either vein of such Hx rats caused significant increases in body weight gain, but the EJV route was almost 3 times more effective than was the HPV (Fig. 5). Pulsed delivery stimulated significant weight gain only when the hGH was given into the EJV, but it was only about half as effective as was constant infusion into that vessel.

The data on TEPW measurements with hGH infusion are consistent with the body-weight changes (Fig. 5). Infusion into the EJV was more effective than delivery into the HPV by either mode of delivery, and constant infusion into the EJV or the HPV was significantly more effective than pulsed delivery into either vein.

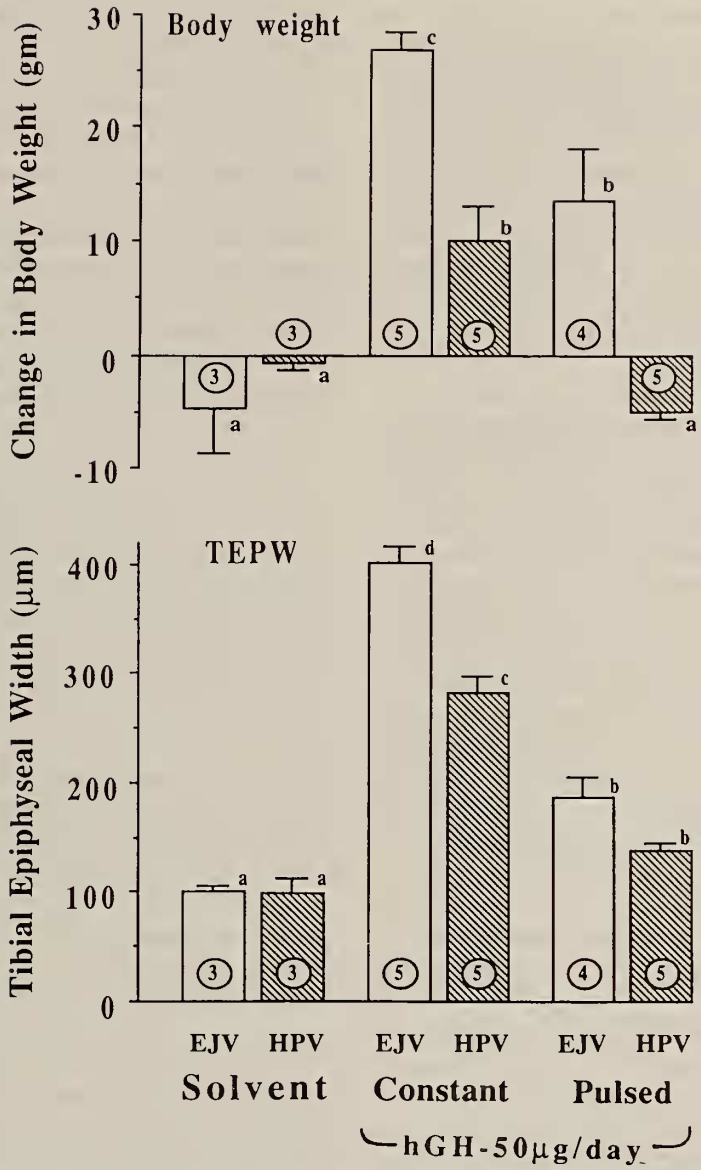

FIG. 5. Effects of infusing hGH (50 μg/rat/day) for 7 days on body weight gain and tibial epiphysial plate width of rats that were Hx 30 days previously. Otherwise as in the legend in Fig. 1. Columns with different letters are significantly different at $P<0.05$ or better.

DISCUSSION

The results presented in this paper show that delivery of a low dose of rat GH directly to the liver via its portal blood supply was no more effective at maintaining growth or elevating serum IGF-I concentration than was infusion at a distant site, regardless of whether the hormone was given constantly or in pulses (Figs. 1 and 2). These findings were unexpected because the liver is generally thought to be the major source of GH-stimulated IGF-I secretion [2, 3], and intrahepatic infusion should have exposed the organ to a concentration of GH that was at least 4 times higher

than that achieved by infusion into the EJV [see 22]. Administration of a higher daily dose of either bGH or hGH gave results that were even more surprising. Infusion of these hormones into the EJV was significantly more effective at stimulating all of the growth parameters measured than was delivery directly into the liver, regardless of whether they were infused constantly or in pulses. Thus, none of these experiments provide data to support the idea that stimulation of hepatic IGF-I secretion by GH is the major means by which the hormone promotes growth.

Another surprising aspect of our studies was the finding that constant infusion of GH was either as effective (with rGH) or more effective (bGH, hGH) than was pulsatile delivery. Several groups have reported that pulsed infusion [23–25] or frequent injections [26–28] of GH are more effective at restoring growth in Hx rats than is constant infusion. However, in one of these studies [23], pulsatile delivery was not more effective than constant infusion during the first week of the treatment; in other experiments [27, 28], the frequency of injections was not related to the magnitude of growth responses until after 5–7 days of treatment. The schedule that we used corresponds to the period when these investigators [23, 27, 28] found that intermittent delivery was not more effective than continuous treatment. Nevertheless, this does not explain our finding that constant infusion of bGH and hGH was more effective than giving pulses of 1-hr duration, while none of the other studies found this effect during the first few days of treatment, and one of them [25] found the opposite result.

By measuring the flow of air bubbles and solvent segments in the catheters, we determined that the minipumps did perform, on average, as expected in terms of delivering the pulses according to the schedule selected [29]. However, there was considerable variation in pulse duration and the interpulse interval. We were unsuccessful in determining the pattern of GH in the blood of the infused animals because of difficulties in collecting a sufficient number of samples serially. Nevertheless, a major difference between our study and those of the others who infused the GH in pulses was in the duration of the pulses. While our system delivered the hormone in square-wave pulses of about 1 hr duration, the other groups used pulse intervals of only a few minutes.

In separate experiments we have investigated the effectiveness of constant vs. pulsed delivery of ovine PRL at restoring lactation in rats in which endogenous PRL secretion was suppressed by bromocriptine [29]. Constant infusion was again found to be consistently more effective than any of several pulse infusion schedules [29], but the difference was not as striking as we observed here with hGH and bGH. In that experiment [29] and in another study [30] we also found that intrahepatic infusion of the PRL was not more effective at restoring lactation than was infusion into the EJV. It is unlikely that downregulation or desensitization of the hepatic GH receptors can account for the lesser effectiveness of intrahepatic *vs* intrajugular delivery of the hormone as constant delivery should be more effective than pulsed infusion at reducing hepatic responsiveness to GH.

When PRL was infused into the HPV of pigeons [15] or rats [31], it was more effective at stimulating growth of target organs of PRL than was infusion of the same dose of the hormone into the EJV. Likewise, infusing insulin into the HPV of diabetic rats was more effective at promoting body growth and normalizing their metabolic status than was delivery into the EJV [32]. It has also been shown that direct delivery of PRL to the liver of bullfrog tadpoles (*Rana catesbeiana*) was more effective at promoting growth and inhibiting metamorphosis than was delivery to the kidney or subcutaneously [33]. These results with PRL [15, 31, 33] and insulin [32] indicate that if GH did have a preferential effect on the liver to promote IGF-I secretion and growth, our infusion system should have allowed it to be manifested. Accordingly, it seems likely that direct hepatic mediation of the growth-promoting effects of GH may be of less significance than is generally considered [1, 2]. Thus, the direct growth-promoting effect of GH on peripheral tissues, which has been well established by recent studies [8–14], may be of greater import for growth than are the hepatic effects of the hormone. This conclusion is supported by the data which show that although intrahepatic delivery of bGH was almost as effective as infusion into the

EJV at elevating serum IGF-I levels (Fig. 4), the HPV route was much less effective at stimulating growth responses (Figs. 4 and 5).

This conclusion does not mean that an effect of GH on the liver is unimportant for growth. Indeed, it has been well established that GH can stimulate IGF-I secretion by liver cells both *in vivo* and *in vitro* [see 3]. However, the significance of the hepatic receptors for GH and the effects that GH may have on liver functions related to growth *via* these receptors may be in maintaining hepatic responsiveness to other factors that can stimulte IGF-I secretion, such as insulin [32] and thyroid hormones [34].

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